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The Toxicity of Paraquat

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Samples of paraquat dichloride and paraquat dimethosulphate are equitoxic when the LD50 is expressed as mg. paraquat ion/kg. body-weight. There are wide species variations in the LD50 and, of course, variations according to the route of administration in a single species.

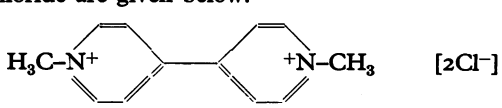
The pathological lesions attributable to paraquat are described in some detail. Among the most unusual is a peculiar proliferative condition in the lungs, which in an extreme case and in many parts can hardly be recognized as consisting of pulmonary tissue. With slight variations, the same microscopical picture may be seen in the rat, mouse, dog, and man, and less often in the rabbit. The experimental evidence suggests that once the condition is initiated it often proceeds in the absence of further exposure to paraquat until it becomes lethal.

There is evidence that much of the mortality resulting from dermal application of paraquat in the rabbit is caused not by percutaneous absorption but by oral contamination from the stratum corneum. This leads to glossitis and oesophagitis and an inability or unwillingness to eat.

In this paper we report the effects of paraquat administered to laboratory animals as the dichloride or dimethosulphate, either as a single dose by one or other route, or over a short term of repeated doses. Observations made by others on the effects of very exceptional cases of poisoning in man, arising from accidental imbibition of a solution of the substance rather than from its use in the field, suggest that similar pathological changes may be expected in human beings.

Chemical Constitution and Uses

Paraquat is a dipyridylium compound. The formula and chemical nomenclature of the dichloride are given below.



1,1′-dimethyl-4,4′-dipyridylium dichloride

One main use of the dichloride and dimethosulphate is as weed-killers. They are effective only if sprayed on to the leaves of plants (more so in light than in the dark), so that by judicious management weeds may be eradicated while adjacent taller plants remain unaffected. They also destroy the foliage of crops such as cotton and potatoes, thus rendering simpler their harvest by modern techniques. In contact with the soil they are rapidly inactivated and are no longer available to plants. They can therefore be used for the control of weeds in a great variety of row-crops and have extensive application in pasture renovation. Their use can eliminate the need for ploughing in the cultivation of annual crops. Consideration of their physico-chemical characteristics suggests that they are dissociated at all *p*H values; the paraquat ion alone, therefore, should determine the toxic effects, and in fact this is the case.

Methods

Usually we used samples of paraquat dichloride or dimethosulphate, some of them 99·9% pure, dissolved in water or isotonic saline and administered by a variety of routes to animals which had free access to food and water. On occasion we incorporated the substance in the diet, from which we could achieve quantitative recovery. In the studies on the effects of paraquat on the skin, however, we used a commercial preparation such as is employed in the field. In subacute toxicity tests, we attempted to examine histopathologically every organ in the body, including not fewer than five levels of the alimentary canal.

When administered in the food, we dissolved the salt of paraquat in water and mixed it with the usual diet

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obtained from the manufacturers in the form of a powder. After adding 20% extract of malt, we extruded the diet through a sausage-meat machine and dried it in a vacuum-oven. We prepared a control diet similarly, except that we did not add a salt of paraquat.

When applied to the skin, the toxicity of paraquat is complicated by several factors best considered in the description of the results obtained. We followed a standard technique throughout. After clipping the backs of the animals on the day before beginning the test, avoiding of course any damage to the skin, we applied the material in a volume of 1 ml./kg. body-weight. As this volume remained constant, the concentration of paraquat in mg./ml. is equal to the dose rate in mg./kg.

We calculated LD50s and the confidence limits by the moving-average interpolation method (Thompson, 1947). Throughout this paper they appear as mg. paraquat ion/kg. body-weight.

Results

Individual animals seemed to differ widely in their response to paraquat, and after both a single dose and repeated doses deaths were strung out over a rather unusually long period. Dosing of animals fasted overnight probably produced more severe effects, but again complete uniformity between experiments did not result. This was so even with a single specimen of the compound. Many animals died between the second and fifth days, after which there were sometimes few deaths until around the tenth to twelfth days. These facts rendered estimation of the LD50 less simple than usual. In Table I the figures for a single dose appear as mg. paraquat ion/kg. body-weight (P = 0·05 confidence limits in parentheses).

At a level of 0·025% paraquat ion in the diet, rats sickened and died within 27 to 57 days; females appeared to be more susceptible and died earlier than males. Both sexes tolerated a level of dosage of 0·01% for many months.

Instillation into the Conjunctival Sac in Rabbits We placed one drop of various concentrations of an aqueous solution (up to 50%) of the dichloride in the conjunctival sac of the rabbit; the other eye served as a control. No initial irritation was evident, but mild inflammation of the conjunctiva and nictitating membrane followed within 12 hours and persisted for 48 to 96 hours. No corneal damage resulted. The 'no-effect' level appeared to be above 0·286 g./100 ml., which is nearly twice the normal working strength of the solution.

Cutaneous Toxicity in the Rabbit Our observations tended to show that the precise technique of application used was of more than ordinary importance. The toxicity of an aqueous solution of paraquat applied to an area of skin exposed to the air was lower than when we covered the site of application by an occlusive dressing. Under the latter conditions, with higher daily doses of the compound (5·0 to 6·25 mg. ion/kg. body-weight) the skin became moist and reddened, and the superficial layers sloughed. Amounts as low as 1·56 mg. ion/kg. body-weight led to lesser changes in the skin. It was quite obvious that results obtained with a damaged skin of this nature were hardly applicable to single or occasional exposure in the field, quite apart from the fact that, apparently, the skin of the rabbit is approximately three times more permeable to ions than is human skin (Tregear, 1964). With daily applications for 20 days, the LD50 appeared to be 4·5 mg. ion/kg. body-weight/day.

We applied a watery solution of paraquat (dichloride or dimethosulphate) to about 10% of the animal's body surface which had been clipped the day before. We allowed the solution to dry and prevented licking by applying plastic collars approximately 18 cm. in diameter. After 24 hours we

TABLE I

LD50 OF PARAQUAT IN VARIOUS ANIMAL SPECIES

Animal	*Number per Group*	*Weight (g.)*	*Route of Administration*	*LD50*
Female rats	6	130-160	'Oral'	112 (104-122)
Female rats	10	150-205	'Oral'	150 (139-162)
Female rats*	6	130-160	'Oral'	141 (140-142)
Female rats	6	130-160	Intraperitoneal	19 (16-21)
Female rats*	6	130-160	Intraperitoneal	16 (14-19)
Female guinea-pigs	3	400-500	Intraperitoneal	3
Male guinea-pigs	5	190-250	'Oral'	30 (22-41)
Rhode Island hens	5	1950-3000	'Oral'	262 (200-346)
Female cats	3	2500-4400	'Oral'	35 (27-46)

*The tests were carried out with 99·9% pure paraquat dichloride except for those marked with an asterisk where 99·9% pure dimethosulphate was used. The LD50s are in mg. of paraquat ion/kg. body-weight. The figures in parentheses are the confidence limits (P = 0·05). LD50s when paraquat is applied to the skin of rabbits are considered in the text.

washed the area of application with water and dried it carefully. We then removed the collar. In subacute tests applications were continued for 20 days, and we observed the animals for a further two weeks. An approximate LD50 for a single application was 236 mg. ion/kg. body-weight, and the LD50 in the subacute test was 7 to 14 mg. ion/kg. body-weight/day. Neither clinical nor pathological abnormalities were apparent after repeated applications of 2·8 mg. ion/kg., more than twice the normal working concentration of 0·12%.

Signs of Poisoning after Intraperitoneal Dosing After a single large intraperitoneal dose (30 to 75 mg. ion/kg.) in rats, the signs of poisoning varied somewhat from animal to animal: most pointed to an action of the substance on the central nervous system. In the earlier stages, hyperexcitability, violent forced movements flinging the animal about the cage, or a stiff and incoordinate gait might be present. Spasms might occur, or the limbs might be widely splayed. A rolling gait might continue up to the time of death which, at the levels of dosage employed, usually occurred on or before the fifth day. Additionally, in the earlier stages breathing might be gasping or, alternatively, deep and fast. Some days after a dose respiration became increasingly laboured. Over-secretion of the harderian glands was usually a feature.

In subacute experiments, tremor and hyperaesthesia, and less frequently excessive harderian secretion, might be apparent during and soon after the period of dosing. In animals which survived for a number of days after the last of a series of ascending doses, the later signs of illness were chiefly difficult breathing, accompanied on occasion by a clicking or similar sound and by soiling of the hair around the mouth and nares by a brownish fluid.

Signs of acute poisoning in the mouse were not dissimilar.

Sick rabbits did not eat or drink: they might be hyperaesthetic or show a creamy exudate around the reddened eyelids. More often than not, however, the animal was dead in its cage one morning without having shown more than inappetence on the previous day. The histological abnormalities in a very limited number of animals were not sufficiently constant or severe to account for death.

Post-mortem Findings after Intraperitoneal or Oral Dosing At necropsy, after a single large or a few smaller doses by whatever route, the lungs might be very congested or show early 'consolidation'; four rabbits examined, however, showed slight or no evidence of severe pulmonary disorder. Some rats had a thymus smaller than normal for their age and a small, pale spleen. Two of the four rabbits examined had small, pale spleens; they were too old to have had a large thymus at the beginning of the experiment.

The rats and mice with laboured respiration four or five days after a single dose, or at the end of a subacute experiment, all showed various degrees of consolidation of the lungs, which usually were plum-coloured and often sank in water. If they floated, there often escaped from a cut surface long red threads which sank to the bottom of the bowl; these were composed of blood which seemed to be more than normally coherent. Sometimes a frothy fluid exuded from the trachea.

After continued dosing with a concentration of 0·025% paraquat ion in the diet, rats began to die with respiratory signs after about 27 days. In some of these animals the appearance of the lungs was that described above. In others there was less congestion, and, instead of being plum-coloured, affected areas of the lung were greyish and fleshy; they gave the impression of being airless and they sank in water.

Histological Appearances

In the Rat, Mouse and Rabbit after Intraperitoneal or Oral Dosing We directed the histological study chiefly to the lesions in the rat. Those in the mouse were reasonably similar, but in only one rabbit did we observe definite changes (other than vascular congestion) in the lungs, changes which were so characteristic a feature in the other species.

The most characteristic lesion found in the rats which died after the administration of paraquat was that in the lungs. We followed all stages of this lesion up to the final state of near-solidity of much of the lung substance. The symptomatology and the microscopical picture both suggested that in animals which died five to nine days after a dose or doses of paraquat, impaired pulmonary function contributed materially to the fatal outcome.

In the majority of animals the lungs were very congested, with oedema fluid in many of the alveoli and an excess of macrophages in others. Perivascular and peribronchial oedema also occurred, particularly in the earlier stages; later, proliferating cells were so numerous that they left no space for fluid in these situations. This cellular proliferation began as early as the third day, and at first the cells appeared as more or less typical fibroblasts arising from the adventitia of the vessels and the fibrous tissue around the bronchi (Fig. 1). Polymorphonuclear leucocytes, some karyorrhectic, mononuclears, and macrophages were present, and in some animals large numbers of eosinophils.

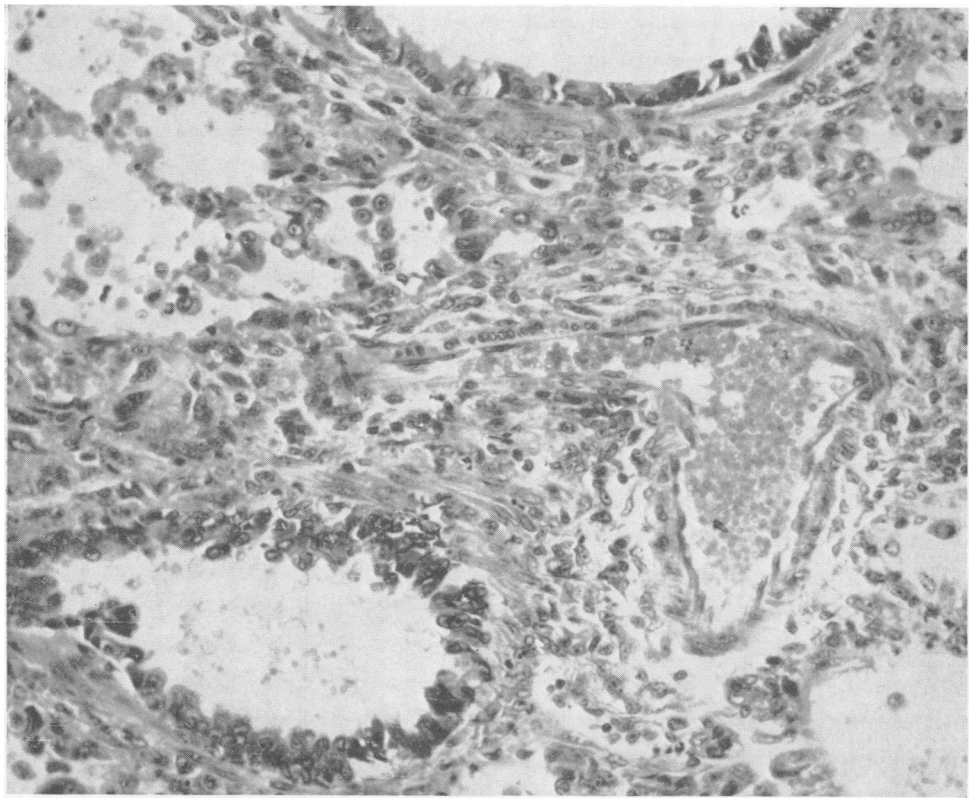

FIG. 1. Proliferation of fibroblasts around the vessels and bronchi in the earlier stages of reaction to paraquat in rats. Haematoxylin and eosin, × 250.

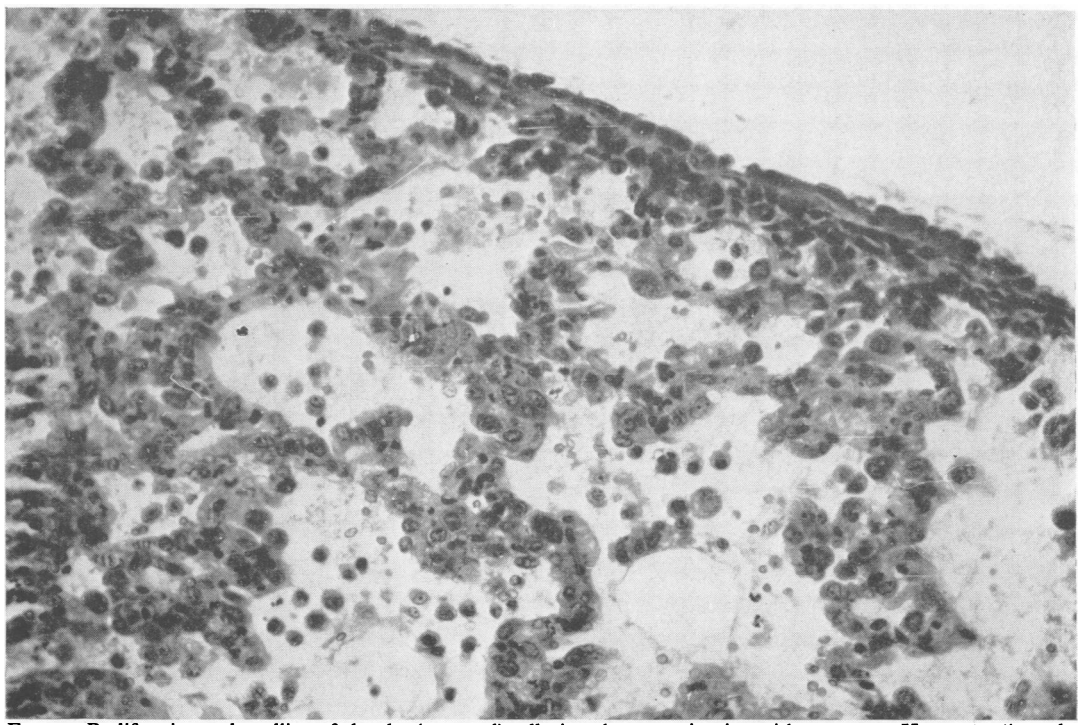

FIG. 2. Proliferation and swelling of alveolar (or septal) cells in subacute poisoning with paraquat. Haematoxylin and eosin, × 250.

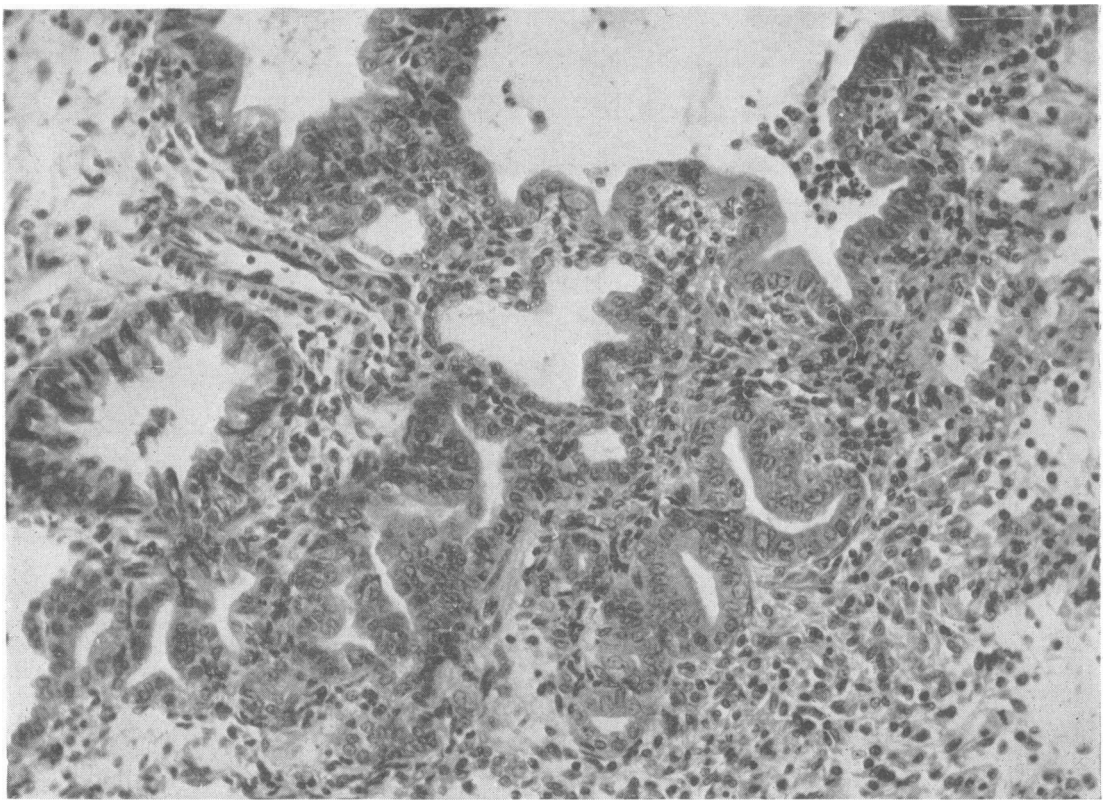

FIG. 3. Proliferation of the epithelium of the terminal bronchi. Haematoxylin and eosin, × 250.

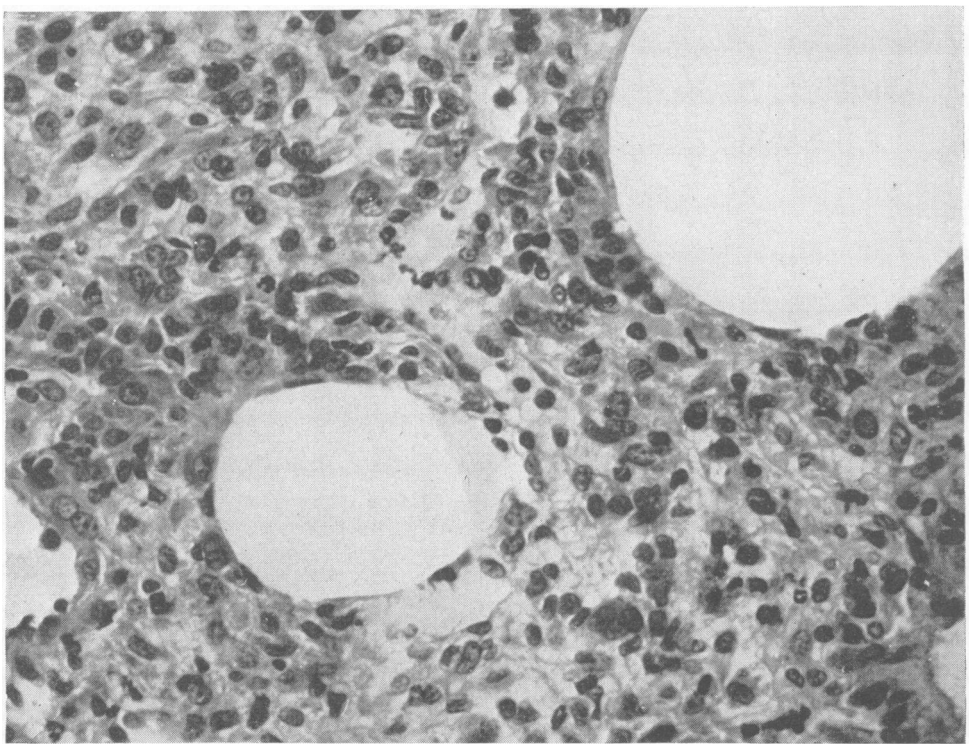

FIG. 4. Most of the lung of this animal was solid and sank in water. Haematoxylin and eosin, × 250.

As the days went by, the cellular proliferation increased and spread into the alveolar walls, where it was both diffuse and accentuated in whorls. The cells were now much smaller and spindle-shaped or elongated and no longer characteristically fibroblastic. Mitoses were very numerous. The epithelial cells of the alveoli (or the septal cells) also participated in the proliferative process (Fig. 2), and some animals showed tiny duct-like structures apparently derived from these cells. In other animals larger spaces were lined by proliferated epithelium from the terminal bronchioles (Fig. 3). The extreme picture was seen in rats on long-term feeding; large areas of the lung were solid and consisted of massed cells with no air-containing cavities between (Fig. 4). Both at this late stage and at an earlier stage with much pulmonary oedema, the remaining air-containing areas of the lungs tended to show distended alveoli and alveolar ducts.

The foregoing describes the appearances in the majority of animals. In some, however, the epithelial proliferation, whether of alveolar cells or of terminal bronchial epithelium, predominated in the absence of great fibroblastic overgrowth. In some animals also, a few giant cells of different types were present. Over a series of animals, therefore, the histological pictures were very diverse.

Once started, the consolidation of the lungs appeared to be progressive in the absence of further dosing with paraquat: in some subacute tests the animals appeared reasonably well at the time dosing ceased, only to develop embarrassed respiration and to die five to seven days later.

By comparison with the lungs, other organs were relatively lightly affected.

The liver was usually that of a fasting animal. There might be some vacuolation of liver cells around the central veins, and a very few isolated necrotic cells here or mid-zonally (five out of 13 rats examined histologically).

The kidney of one rat showed occasional necrotic cells in the proximal convoluted tubules. In 10 of 13 rats a variable number of other tubules, probably the distal convoluted tubules, were lined by greatly swollen cells with 'empty' cytoplasm and pyknotic nuclei. The remains of such cells sometimes lay loose in some of the tubules of Henle.

The adrenals were congested. The thymus (eight out of 13 rats) and the spleen (one out of 12 rats) showed some lympholysis.

In the testis some seminiferous tubules showed degenerative changes, with the formation of small multinucleated giant cells lying free in the lumen (two out of five rats).

Mice which died at six or seven days, after the last of a series of doses, showed a not dissimilar picture in the lungs, but the leucocytic element in the exudate was more pronounced. In many of the larger arteries and veins polymorphs existed in very large numbers; they tended to collect as a peripheral layer several cells deep, leaving the red blood corpuscles free of leucocytes in the central stream. They also occurred in large numbers around some of the vessels and bronchi, in the alveolar walls, and free in the alveoli. The last might suggest an infective process, but the overall picture in a number of animals was quite different. In the most severely affected animal, many polymorphs were karyorrhectic, and in one or two places the alveolar walls seemed to have disintegrated. Around some, but not all, of the blood vessels oedema was marked, and early proliferation of fibroblasts was seen. All the lungs were intensely congested.

Degeneration of occasional renal tubules occurred in the only mouse examined from this point of view and was similar to that already described in the rat.

By contrast with the rat and the mouse, the rabbit displayed much less tendency to involvement of the lungs; indeed, only one of four animals examined showed any sign of cellular proliferation, although the lungs uniformly were greatly congested in these clinically severely affected or moribund animals. Inconstant and not very severe changes included some necrosis in the liver, necrosis of a few cells in the proximal convoluted tubules in the kidney, degeneration of a few renal tubules similar to that observed in rats and mice, necrosis of cells in the outermost zone of the adrenal cortex, a mild degree of testicular degeneration, and some lympholysis in the thymus.

In the Dog Experiments on dogs carried out in the United States by Industrial BIOTEST Laboratories, Inc. led in the lungs of some animals to a condition described as 'fibrosing pneumonitis'. Through the courtesy of Dr. J. C. Calandra we were privileged to see sections from these animals. In our opinion, the condition present was essentially similar to that in our rats fed for a long period with a low concentration of paraquat in the diet.

In Man Three cases of poisoning in man, none 'occupational', were brought to our notice. All followed accidental ingestion of a solution of paraquat. Through the courtesy of Dr. E. E. Doyle and Mr. G. A. McL. Lee we were able to examine the lungs of a patient who died in Ireland, and through the courtesy of Dr. C. M. Bullivant we examined lungs from patients who died in New Zealand. Here again the pulmonary lesions closely resembled those in the rats fed paraquat. The precise amount of paraquat ingested and retained in the body in

these cases was difficult to ascertain, but it might have been several hundred milligrams per kilogram body-weight.

Signs of Poisoning and Pathology in the Rabbit after Dermal Application Applied to the skin of the rabbit paraquat led to a different clinical picture. The most striking effect, after a latent period, was the secretion of copious amounts of brownish saliva. Rabbits so affected refused to eat, and death occurred in a state of cachexia. In some of these animals renal tubular damage and pulmonary changes appeared as before. In others, the pulmonary changes were pronounced; the alveolar walls were thickened and contained a mixed cell infiltrate in which plump histiocytes were conspicuous. In a minority the pulmonary changes resembled those in the rat but, although greatly thickened alveolar walls obliterated alveoli and caused consolidation in some areas, there was little evidence at this stage that the initial change was perivascular or peribronchial; the interstitial tissue of the lung was diffusely involved. At low doses the pulmonary changes were often of doubtful significance and in some circumstances could reasonably be accepted as being within normal limits.

At first the significance of the profuse salivation was not recognized, but it was soon evident that this phenomenon was associated with glossitis and even ulceration of the tongue and oesophagus. Oral contamination did not appear to be taking place, but further investigations, not reported here in full, showed that excess salivation and glossitis did not occur when oral contamination was absolutely excluded by leaving the plastic collars in position after washing the sites of application and, in addition, by covering the washed and dried skins with dry porous dressings. Conversely, when the rabbits were given drinking water containing 286 p.p.m. of paraquat dichloride (1:1000 dilution of the commercial preparation supplied) they developed glossitis within two days and then rapidly lost weight.

In order to assess the effect of early decontamination of the skin following exposure to paraquat, we washed the rabbits' skins with water after an application of 280 mg. ion/kg. body-weight so as to give an exposure varying from 15 minutes to four hours. We then returned these rabbits to their cages without their plastic collars. Lesions definitely attributable to paraquat were not apparent either in the lungs or in the kidneys, but glossitis and oesophagitis occurred, and anorexia and loss of weight were pronounced. It was assumed that, even after decontamination, there was a reservoir of paraquat in the skin and that persistent oral contamination occurred through grooming during the period of observation. Consequently, it is believed that following skin application death, in many cases, was due to wasting following an inability to eat, and that this resulted from lingual and oesophageal ulceration or inflammation caused by continued oral contamination from a skin reservoir. Even during the subacute 20-day test animals were exposed daily to the possibility of oral contamination because, after washing and drying the skin, we left them without collars until the time of the next application.

Discussion

For the most part this report concerns the toxicity of paraquat in small laboratory animals, and particularly in the rat and mouse. The pathological features described seem also to be common to poisoning in the dog and in man. Apart from the effect of large doses on the central nervous system, two points merit attention.

One is that often the substance gives rise in the lungs to a peculiar lesion which, betraying at first but little evidence of its presence, progresses in the absence of further dosing to a point incompatible with life. As this condition may result from a single dose of paraquat, and as Daniel and Gage (1966), using the substance labelled with ^{14}C, have found it poorly absorbed and in any case quickly excreted in the urine, we cannot consider the characteristic lesions as due to a cumulative effect of the herbicide. Rather, the compound initiates irreversible changes in the pulmonary tissues, but these are not recognized clinically until they are sufficiently advanced to bring the animal nearly to the point of death. Several analogous phenomena are known in the field of experimental (and human) pathology.

The second point is the behaviour of paraquat when applied to the skin of the rabbit. It is well known that the stratum corneum can act as a skin reservoir for applied substances, as the experiments of Vickers (1964) have so elegantly shown. This is especially probable in the case of paraquat, which is readily adsorbed by other materials. It is thus available for continual application to the tongue during normal grooming, with consequent glossitis and failure to eat. It is unlikely to be available for absorption percutaneously, since the keratinous layer is shed.

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